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Search History

DATE: Monday, January 05, 2004 Printable Copy Create Case

Set Name side by side	Query	Hit Count	Set Name result set
DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI; PLUR=YES; OP=ADJ			
L16	L15 same l14	4	L16
L15	protective	656404	L15
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L13	cpg motif	298	L13
L12	L11 and cpg	0	L12
L11	L10 and l7	26	L11
L10	L9 same l8	27	L10
L9	amphiphile	2174	L9
L8	spermine with cholesterol	108	L8
L7	tumor or cancer	206115	L7
L6	l3 and l2	37	L6
L5	l3 same l2	9	L5
L4	L3 same l2	9	L4
L3	cpg with plasmid	111	L3

L2	cationic amphiphile or cationic lipid or cationic liposome	8019	L2
L1	CpG with cationic	25	L1

END OF SEARCH HISTORY

L6: Entry 33 of 37 File: USPT Apr 17, 2001

DOCUMENT-IDENTIFIER: US 6218371 B1
TITLE: Methods and products for stimulating the immune system using immunotherapeutic oligonucleotides and cytokines

Detailed Description Text (62):
Lipid formulations for transfection are commercially available from QIAGEN, for example as EFFECTENE.TM. (a non-liposomal lipid with a special DNA condensing enhancer) and SUPER-FECT.TM. (a novel acting dendrimeric technology) as well as Gibco BRL, for example, as LIPOFECTIN.TM. and LIPOFECTACE.TM., which are formed of cationic lipids such as N-[1-(2,3 dioleyloxy)-propyl]-N,N,N-trimethylammonium chloride (DOTMA) and dimethyl dioctadecylammonium bromide (DDAB). Methods for making liposomes are well known in the art and have been described in many publications. Liposomes were described in a review article by Gregoriadis, G., Trends in Biotechnology 3:235-241 (1985), which is hereby incorporated by reference.

Detailed Description Text (79):
Preferably the CpG oligonucleotide is in the range of between 8 and 100 and more preferably between 8 and 30 nucleotides in size. Alternatively, CpG oligonucleotides can be produced on a large scale in plasmids and degraded into oligonucleotides.

Detailed Description Text (80):
The CpG oligonucleotide and immunopotentiating cytokine may be directly administered to the subject or may be administered in conjunction with a nucleic acid delivery complex. A "nucleic acid/cytokine delivery complex" shall mean a nucleic acid molecule and/or cytokine associated with (e.g. ionically or covalently bound to; or encapsulated within) a targeting means (e.g. a molecule that results in higher affinity binding to target cell (e.g. dendritic cell surfaces and/or increased cellular uptake by target cells). Examples of nucleic acid/cytokine delivery complexes include nucleic acids/cytokines associated with: a sterol (e.g. cholesterol), a lipid (e.g. a cationic lipid, virosome or liposome), or a target cell specific binding agent (e.g. a ligand recognized by target cell specific receptor). Preferred complexes should be sufficiently stable in vivo to prevent significant uncoupling prior to internalization by the target cell. However, the complex should be cleavable under appropriate conditions within the cell so that the nucleic acid/cytokine is released in a functional form.

L11: Entry 20 of 26 File: USPT Nov 24, 1998

DOCUMENT-IDENTIFIER: US 5840710 A
**** See image for Certificate of Correction ****
TITLE: Cationic amphiphiles containing ester or ether-linked lipophilic groups for intracellular delivery of therapeutic molecules

Brief Summary Text (6):
One of the fundamental challenges now facing medical practicioners is that although the defective genes that are associated with numerous inherited diseases (or that represent disease risk factors including for various cancers) have been isolated and characterized, methods to correct the disease states themselves by providing patients with normal copies of such genes (the technique of gene therapy) are substantially lacking. Accordingly, the development of improved methods of intracellular delivery therefor is of great medical importance.

Brief Summary Text (7):
Examples of diseases that it is hoped can be treated by gene therapy include inherited disorders such as cystic fibrosis, Gaucher's disease, Fabry's disease, and muscular dystrophy. Representative of acquired disorders that can be treated are: (1) for cancers--multiple myeloma, leukemias, melanomas, ovarian carcinoma and small cell lung cancer; (2) for cardiovascular conditions--progressive heart failure, restenosis, and hemophilias; and (3) for neurological conditions--traumatic brain injury.

Brief Summary Text (29):
In one preferred embodiment, the steroid component "Z" is selected from the group consisting of 3-sterols, wherein said sterol molecule is linked by the 3--O-- group thereof, or by N-- in replacement thereof, to Y (or directly to X, if Y is absent). According to this aspect of the invention, particularly effective amphiphiles include, for example, spermidine cholesterol carbamate (N.sup.4 -spermidine cholesteryl carbamate, amphiphile No. 53), and spermine cholesterol carbamate (N.sup.4 -spermine cholesteryl carbamate, amphiphile No. 67), and amphiphiles patterned thereon.

Detailed Description Text (10):
As examples of the cationic amphiphiles of the invention, both spermidine cholesterol carbamate (N.sup.4 -spermidine cholesteryl carbamate) and spermine cholesterol carbamate (N.sup.4 -spermine cholesteryl carbamate) have been determined to be superior transfectants in vivo in comparison with non "T-shaped" amphiphiles having otherwise equivalent amounts of cationic alkylamine structure. Superior performance (see also Example 3) has been determined for: ##STR9## in comparison with, for example, ##STR10##

Detailed Description Text (12):
Applicants have also noted that numerous of the cationic amphiphiles of the invention have structural features in common with naturally occurring polyamines such as spermine and spermidine (including N-atom spacing). In this regard, the structures of amphiphiles 53, 67, 78, 90, and 91 are representative. As can be seen by examination of the data in FIGS. 13, 14 and 15, the placement of the nitrogen atoms in the polar head groups of the amphiphiles such that they are separated by one or more combinations of 3 and 4 carbon atoms leads to high in vivo transfection

efficiency for plasmid transgenes complexed therewith. Applicants have also noted that these in-common structural features may have a useful effect upon the binding of the amphiphiles to DNA, and on interaction with cell surface polyamine receptors. Interaction with cell polyamine receptors may be particularly important with respect to the treatment of cancer cells by gene therapy, since the DNA replication requirements of such cells may lead to high level expression of such receptors.

Detailed Description Text (30):
As elaborated below, certain preferred amphiphiles of the invention include a steroid component "Z" that is selected from the group consisting of 3-sterols, wherein said sterol molecule is linked by the 3-O-group thereof, or by N- in replacement thereof, to Y (see FIG. 1). Such structures include, for example, spermidine cholesterol carbamate, spermine cholesterol carbamate, spermidine 7-dehydrocholesteryl carbamate, lysine 3-N-dihydrocholesteryl carbamate, spermidine cholestamine urea, and N-3-aminopropyl-N-4-aminobutylcholestamine.

Detailed Description Text (94):
Although heretofore unrecognized in the art, it has been determined also that certain co-lipids may react chemically with certain types of cationic amphiphiles under conditions of co-storage, there resulting new molecular species. Generation of such new species is believed to occur via mechanisms such as transacylation. In this regard, see FIG. 4 which depicts a transacylation reaction involving spermine cholesterol carbamate(No.67) and DOPE, there resulting lyso PE species and multiple forms of particular acyl-cationic amphiphile (designated No. 80).

Detailed Description Text (99):
The present invention provides for pharmaceutical compositions that facilitate intracellular delivery of therapeutically effective amounts of biologically active molecules. Pharmaceutical compositions of the invention facilitate entry of biologically active molecules into tissues and organs such as the gastric mucosa, heart, lung, and solid tumors. Additionally, compositions of the invention facilitate entry of biologically active molecules into cells that are maintained in vitro , such as in tissue culture. The amphiphilic nature of the compounds of the invention enables them to associate with the lipids of cell membranes, other cell surface molecules, and tissue surfaces, and to fuse or to attach thereto. One type of structure that can be formed by amphiphiles is the liposome, a vesicle formed into a more or less spherical bilayer, that is stable in biological fluids and can entrap biological molecules targeted for intracellular delivery. By fusing with cell membranes, such liposomal compositions permit biologically active molecules carried therewith to gain access to the interior of a cell through one or more cell processes including endocytosis and pinocytosis. However, unlike the case for many classes of amphiphiles or other lipid-like molecules that have been proposed for use in therapeutic compositions, the cationic amphiphiles of the invention need not form highly organized vesicles in order to be effective, and in fact can assume (with the biologically active molecules to which they bind) a wide variety of loosely organized structures. Any of such structures can be present in pharmaceutical preparations of the invention and can contribute to the effectivenesss thereof.

Detailed Description Text (116):
An additional aspect of the invention concerns the protonation state of the cationic amphiphiles of the invention prior to their contacting plasmid DNA in order to form a therapeutic composition. It is within the practice of the invention to utilize fully protonated, partially protonated, or free base forms of the amphiphiles in order to form such therapeutic compositions. With respect to amphiphile No. 67 (spermine cholesterol carbamate), it has been observed that when providing this amphiphile for a transfecting composition with DOPE (itself provided as a zwitterion), transgene expression was best for the free base, but decreased if the amphiphile was prepared as an acetate salt. Activity decreased step-wise

through the mono and di acetate salts and was minimal for the triacetate salt. Under the circumstances described, the plasmid DNA provided for contacting with the amphiphile was prepared (without buffer) as a sodium salt in water.

Detailed Description Text (195):
Experiments were also performed using spermine cholesterol carbamate (amphiphile No. 67) and other amphiphiles of the invention. With respect to spermine cholesterol carbamate, the optimum molar ratio of amphiphile to DOPE under the conditions tested was determined to be 1:2, not 1:1. Optimized ratios for many of the amphiphiles of the invention are reported in FIGS. 13, 14 and 15, and are readily determined by those skilled in the art.

Detailed Description Text (227):
The in vivo data reported in FIGS. 13, 14 and 15 were compiled generally as follows. As aforementioned, FIGS. 10 and 11 report data from the complete in vivo optimization of amphiphile No. 53. Amphiphile No. 67 was subjected to a similar partial optimization. With respect to all of the other cationic amphiphiles reported on, and taking advantage of numerous structural similarities, optimized compositions for in vivo testing were extrapolated from in vitro results. This facilitated the screening of large numbers of amphiphiles and produced broadly, if not precisely, comparable data. For all amphiphiles other than Nos. 53 and 67, the ratio, for in vivo testing, of amphiphile concentration to DOPE concentration was taken from the in vitro experiments, as was the optimized ratio of amphiphile concentration to DNA concentration (see Example 1). Accordingly, for such amphiphiles the in vivo test concentration was fixed at 1 mM, thereby fixing also the co-lipid concentration. [Broadly, the molar ratio of the amphiphile to co-lipid DOPE ranged from 1:2 (for example, spermine cholesterol carbamate, No. 67) through 1:1 (for example, spermidine cholesterol carbamate, No. 53) to about 2:1 (for example, amphiphile No. 75)]. The concentration of plasmid DNA varied for each amphiphile species tested in order to duplicate the optimized amphiphile/DNA ratio that had been determined in vitro.

Detailed Description Text (230):
Also following the procedures of Example 3, part B, and using respectively 4 mM (as nucleotide), 1 mM, and 2 mM concentrations of DNA, amphiphile and co-lipid, the transfection enhancement provided by spermine cholesterol carbamate (amphiphile No. 67)--in relation to N.sup.1 -thermospermine cholesteryl carbamate and N.sup.1 - spermine cholesteryl carbamate to which spermine cholesterol carbamate is similarly related--is at least about 30 fold.

Detailed Description Text (260):
Following generally the procedures described in Example 1, a thin film (evaporated from chloroform) is produced wherein spermine cholesterol carbamate (amphiphile No. 67) and DOPE are present in the molar ratio of 1:2. The amphiphile-containing film is rehydated in water-for-injection with gentle vortexing to a resultant amphiphile concentration of about 3 mM. However, in order to increase the amount of amphiphile/DNA complex that may be stably delivered by aerosol as a homogeneous phase (for example, using a Puritan Bennett Raindrop nebulizer from Lenexa Medical Division, Lenexa, Kans., or the PARI LC Jet.TM. nebulizer from PARI Respiratory Equipment, Inc., Richmond, Va.), it may be advantageous to prepare the amphiphile-containing film to include also one or more further ingredients that act to stabilize the final amphiphile/DNA composition. Accordingly, it is presently preferred to prepare the amphiphile-containing film as a 1:2:0.05 molar mixture of amphiphile No. 67, DOPE, and PEG.sub.(5000) -DMPE. [A suitable source of PEG-DMPE, polyethylene glycol 5000--dimyristoylphoshatidyl ethanolamine, is Catalog No. 880210 from Avanti Polar Lipids, Alabaster, AL]. Additional fatty acid species of PEG-PE may be used in replacement therefor.

Detailed Description Text (279):
Chronic inflammation is associated with numerous of the disease states that can be

treated by gene therapy. Representative of such disease states are cystic fibrosis (using CFTR), bronchitis, adult respiratory distress syndrome (using alpha-1 antitrypsin), and metastatic cancers (through upregulation of p53, TIMP-1, and TIMP-2). Inflammatory conditions typically involve many interrelated processes (for example, involvement by many types of immune system cells and liver proteins), whereby the body attempts to heal a damaged or infected tissue. However, chronic inflammation which persists as a result of an unresolved condition may lead to permanent tissue damage, as is the case with respect to lung tissue affected by cystic fibrosis and associated and unresolved lung infections. In fact, permanent damage to the lung tissue of cystic fibrosis patients is a leading cause of their mortality. It would be desirable to provide gene therapy in such a manner as to treat inflammatory conditions associated with the targeted disease state.

Detailed Description Text (283):
It has been determined that numerous biologically active molecules are present in tissues at concentrations thereof that increase with the severity of an inflammatory condition (for example, tumor necrosis factor "TNF" and potentially transcription factors such as NF-kB, AP-1, NF-IL6 and octamer binding protein). It has also been determined that interleukin 8, a polypeptide of 8,500 MW, is upregulated by inflammation and acts as a potent chemoattractant for T lymphocytes and neutrophil cells that are themselves involved in the inflammation response. The interleukin 8 gene is regulated primarily at the transcriptional level, and it has also been determined (H. Nakamura et al., Journal of Biological Chemistry, 266,19611-19617,1991) that TNF can increase interleukin 8 transcription by more than 30-fold in vitro in bronchial epithelial cells. Accordingly, there follows description of gene therapy vectors which take advantage of the above.

Other Reference Publication (22):
A. Pegg, "Polyamine Metabolism and its Importance in Neoplastic Growth and as a Target for Chemotherapy", Cancer Research, 48, 1988, pp. 759-774.

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L11: Entry 26 of 26 File: DWPI Jan 21, 1999

DERWENT-ACC-NO: 1999-120520
DERWENT-WEEK: 199924

TITLE: In vivo transfection of tissue with retinoblastoma gene - formulated with spermine cholesterol carbamate as cationic amphiphile to facilitate transport, used for gene therapy of cancers and tumours

Basic Abstract Text (1):
Transfection of tissues in vivo comprises administering to the patient's vascular or lymphatic systems, or tissue fluid, a composition (A) comprises: (i) DNA encoding retinoblastoma protein (Rb), and (ii) spermine cholesterol carbamate (SSC) as cationic amphiphile, so that (A) contacts the target tissue.

Basic Abstract Text (2):
USE - (A) is used for treatment of cancer and other tumours, particularly (small cell) lung , liver, kidney and ovarian tumours.

Standard Title Terms (1):
VIVO TRANSFECTED TISSUE GENE FORMULATION SPERMINE CHOLESTEROL CARBAMATE CATION AMPHIPHILIC FACILITATE TRANSPORT GENE THERAPEUTIC CANCER TUMOUR

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L11: Entry 25 of 26 File: USPT Jul 22, 1997

DOCUMENT-IDENTIFIER: US 5650096 A
**** See image for Certificate of Correction ****
TITLE: Cationic amphiphiles for intracellular delivery of therapeutic molecules

Detailed Description Text (18):
As elaborated below, certain preferred amphiphiles of the invention include a steroid component "Z" that is selected from the group consisting of 3-sterols, wherein said sterol molecule is linked by the 3--O-- group thereof, or by N-- in replacement therof, to Y. See FIGS. 1 and 4. Such structures include, for example, spermidine cholesterol carbamate (See FIG. 1), spermine cholesterol carbamate (see FIG. 2), spermidine 7-dehydrocholesteryl carbamate (FIG. 3), lysine 3-N-dihydrocholesteryl carbamate (FIG. 4), spermidine cholestamine urea (FIG. 5), and N-3-aminopropyl-N-4-aminobutylcholestamine (FIG. 6).

Other Reference Publication (23):
A. Pegg, "Polyamine Metabolism and its Importance in Neoplastic Growth and as a Target for Chemotherapy", Cancer Research, 48, 1988, pp. 759-774.

CLAIMS:

5. The cationic amphiphile spermine cholesterol carbamate, ##STR8##

L16: Entry 4 of 4 File: USPT Jan 15, 2002

DOCUMENT-IDENTIFIER: US 6339068 B1
**** See image for Certificate of Correction ****
TITLE: Vectors and methods for immunization or therapeutic protocols

Detailed Description Text (38):
The term "effective amount" of a nucleic acid molecule refers to the amount necessary or sufficient to realize a desired biologic effect. For example, an effective amount of a nucleic acid construct containing at least one unmethylated CpG for treating a disorder could be that amount necessary to induce an immune response of sufficient magnitude to eliminate a tumor, cancer, or bacterial, parasitic, viral or flngal infection. An effective amount for use as a vaccine could be that amount useful for priming and boosting a protective immune response in a subject. The effective amount for any particular application can vary depending on such factors as the disease or condition being treated, the particular nucleic acid being administered (e.g. the number of unmethylated CpG motifs (-S or -N) or their location in the nucleic acid), the size of the subject, or the severity of the disease or condition. One of ordinary skill in the art can empirically determine the effective amount of a particular oligonucleotide without necessitating undue experimentation. An effective amount for use as a prophylactic vaccine is that amount useful for priming and boosting a protective immune response in a subject.

Detailed Description Text (39):
In one embodiment, the invention provides a nucleic acid construct containing CpG motifs as described herein as a pharmaceutical composition useful for inducing an immune response to a bacterial, parasitic, fingal, viral infection, or the like, or to a tumor in a subject, comprising an immunologically effective amount of nucleic acid construct of the invention in a pharmaceutically acceptable carrier. "Administering" the pharmaceutical composition of the present invention may be accomplished by any means known to the skilled artisan. By "subject" is meant any aninal, preferably a mammal, most preferably a human. The term "immunogenically effective amount," as used in describing the invention, is meant to denote that amount of nucleic acid construct which is necessary to induce, in an animal, the production of a protective immune response to the bacteria, fungus, virus, tumor, or antigen in general.

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L6: Entry 16 of 37 File: PGPB Mar 13, 2003

DOCUMENT-IDENTIFIER: US 20030050261 A1
TITLE: Immunostimulatory nucleic acid molecules

Brief Description of Drawings Paragraph:
[0031] FIG. 5 is a bar graph plotting chloramphenicol acetyltransferase (CAT) activity in WEHI-231 cells transfected with a promoter-less CAT construct (pCAT), positive control plasmid (RSV), or IL-6 promoter-CAT construct alone or cultured with CpG 5' TCCATGACGTTCCTGATGCT 3' (SEQ ID NO:7) or non-CpG 5' TCCATGAGCTTCCTGAGTCT 3' (SEQ ID NO:8) phosphorothioate ODN at the indicated concentrations. Data present the mean of triplicates.

Detail Description Paragraph:
[0057] For economic reasons, preferably the immunostimulatory CpG DNA is in the range of between 8 to 40 base pairs in size if it is synthesized as an oligonucleotide. Alternatively, CpG dinucleotides can be produced on a large scale in plasmids, which after being administered to a subject are degraded into oligonucleotides. Preferred immunostimulatory nucleic acid molecules (e.g. for use in increasing the effectiveness of a vaccine or to treat an immune system deficiency by stimulating an antibody [humoral] response in a subject) have a relatively high stimulation index with regard to B cell, monocyte and/or natural killer cell responses (e.g. cytokine, proliferative, lytic or other responses).

Detail Description Paragraph:
[0061] A "nucleic acid delivery complex" shall mean a nucleic acid molecule associated with (e.g. tonically or covalently bound to; or encapsulated within) a targeting means (e.g. a molecule that results in higher affinity binding to target cell (e.g. B-cell and natural killer (NK) cell) surfaces and/or increased cellular uptake by target cells). Examples of nucleic acid delivery complexes include nucleic acids associated with: a sterol (e.g. cholesterol), a lipid (e.g. a cationic lipid, virosome or liposome), or a target cell specific binding agent (e.g. a ligand recognized by target cell specific receptor). Preferred complexes must be sufficiently stable in vivo to prevent significant uncoupling prior to internalization by the target cell. However, the complex should be cleavable under appropriate conditions within the cell so that the nucleic acid is released in a functional form.

Detail Description Paragraph:
[0142] When the vaccine is a DNA vaccine at least two components determine its efficacy. First, the antigen encoded by the vaccine determines the specificity of the immune response. Second, if the backbone of the plasmid contains CpG motifs, it functions as an adjuvant for the vaccine. Thus, CpG DNA acts as an effective "danger signal" and causes the immune system to respond vigorously to new antigens in the area. This mode of action presumably results primarily from the stimulatory local effects of CpG DNA on dendritic cells and other "professional" antigen presenting cells, as well as from the costimulatory effects on B cells.

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L6: Entry 21 of 37 File: PGPB Oct 3, 2002

DOCUMENT-IDENTIFIER: US 20020142974 A1
TITLE: IMMUNE ACTIVATION BY DOUBLE-STRANDED POLYNUCLEOTIDES

Detail Description Paragraph:
[0119] Chemical and physical processes may be used for transfection (e.g., calcium phosphate precipitation, cationic lipid, DEAE-dextran, electroporation, microinjection). Alternatively, introduction of double-stranded polynucleotide may occur by intracellular entry by an infectious agent (e.g., bacterium, protozoan, virus), phagocytosis of a cell or infectious agent, replication of a single-stranded virus, oncogenic transformation, or an exogenous or environmental stimulus.

Detail Description Paragraph:
[0162] FRTL-5 cells were grown in 10 cm dishes (D. S. Singer & J. E. Maguire, CRC Crit. Rev. Immunol. 10: 235-257 (1990); S. I. Taniguchi, et al., Mol. Endocrinol. 12: 19-33 (1998); P. L. Balducci-Silano, et al., Endocrinology 139: 2300-2313 (1998); V. Montani, et al., Endocrinology 139: 290-302 (1998); K. Suzuki, et al., Endocrinology 139: 3014-3017 (1998)) to a density of 2.times.10.sup.6 cells. In FIGS. 1A and 1B, FRTL-5 cells were infected with herpes simplex virus (HSV-2) as described (P. R. Krause, et al., J. Exp. Med. 181: 297-306 (1995)), (FIG. 1A, lanes 1-4). Alternatively, they were transfected with 5 .mu.g HSV DNA fragments (FIG. 1A, lane 7), other noted DNAs (FIG. 1B, lanes 3-7), RNA (FIG. 1B, lanes 8, 9) or 54 bp double-stranded oligodeoxynucleotides (ODNs) from Foamy or cytomegalovirus (FIG. 1B, lanes 10, 11) using the cationic lipid LIPOFECTAMINE PLUS (GIBCO BRL, Gaithersburg, Md.) and the manufacturer's protocol. Total RNA was prepared and Northern analysis performed for MHC Class I, MHC Class II, or glyceraldehyde phosphate dehydrogenase (GAPDH) as described (D. S. Singer & J. E. Maguire, CRC Crit. Rev. Immunol. 10: 235-257 (1990); Taniguchi, S. I. et al., Mol. Endocrinol. 12: 19-33 (1998); P. L. Balducci-Silano, et al., Endocrinology 139: 2300-2313 (1998); Suzuki, K. et al., Endocrinology 139: 3014-3017 (1998)) and at either the times noted or 48 hours after treatment. Cationic lipid treatment alone served as a control of the transfection procedure (Mock). In FIG. 1C, FACS analysis of cell-surface Class I and Class II expression induced by DNA or 100 U/ml rat .gamma.IFN 48 hours after treatment.

Detail Description Paragraph:
[0166] Different transfection procedures using cationic lipid (LIPOFECTAMINE), electroporation, and DEAE-dextran also did not alter the results. Also microinjection into the cytoplasm of cells duplicated these results, as measured in individual cells by immunostaining using specific antibodies to MHC class I and class II as described in whole tissues with autoimmune disease (G. F. Bottazzo, et al., Lancet 2: 1115-1119 (1983); I. Todd, et al., Annals. N. Y. Acad. Sci. 475: 241-249 (1986)). There was no correlation with transfection efficiency; thus, under conditions where 100% of cells exhibited increased MHC class I and class II antigen expression (FIG. 1C), transfection efficiency, measured by including 2 .mu.g pGreen Lantern-1 (GIBCO, BRL, Gaithersburg, Md.) and counting green fluorescent proitein expression in cells, was only 10%. Thus, it appears that it is sufficient to introduce the ds nucleic acids into the cytoplasm to have increased MHC gene expression and all phenomena to be detailed in Example 2.

Detail Description Paragraph:
[0171] Transfection and Northern analysis were performed 48 hours after treatment, exactly as in FIG. 1. In FIG. 2A, FRTL-5 cells were transfected with intact, methylated or DNase-treated plasmid, pcDNA3 or pRc/RSV (Invitrogen, Calif.) (lanes 3-8), single-stranded CpG oligodeoxy nucleotides (ODNs) or control ODNs (lanes 9-12), or ss- or ds-phosphorothioate oligonucleotides (S-oligos) (lanes 13-16). Lane 1 contains RNA from non-treated cells and lane 2 from cells subjected to the transfection procedure only, i.e. without nucleic acids being present. In FIG. 2B, various synthetic polymer nucleotides and their duplexes (Pharmacia Biotech Inc., Piscataway, N.J.) were transfected and analyzed (lanes 3-16) as in FIG. 2A. In FIG. 2C, cells were transfected with 5 .mu.g of dsDNA fragments from 24 bp to 1004 bp in length (lanes 3-10) or with indicated amount of 25 bp dsODNs (lanes 12-15) as described above. In FIG. 2C, Class II expression was measured 48 hours later by RT-PCR as described previously (P. L. Balducci-Silano, et al., Endocrinology 139: 2300-2313 (1998); K. Suzuki, et al., Endocrinology 139: 3014-3017 (1998)). Cells treated with 100 U/ml .gamma.IFN for 48 hours were the positive control.

Detail Description Paragraph:
[0185] CpG oligonucleotides were those described (D. M. Klinman, et al., Proc. Natl. Acad. Sci. U.S.A 93:2879-83 (1996)). Methylation of CpG sites in plasmid DNA from pcDNA3, pRc/RSV, and their restriction fragments was by treatment with SssI methylase (New England BioLabs, Beverly, Mass.) at 37.degree. C. for 2 hours. Methylation of CpG motif was confirmed by resistance to BstUI restriction enzyme (New England BioLabs) which recognizes 5'-CGCG-3' motifs. For DNase I digestion, pcDNA3, pRc/RSV and their restriction fragments were treated with DNase I (Promega, Madison, Wis.) at 37.degree. C. for 30 min, then extracted by phenol-chloroform followed by ethanol precipitation. Digestion was confirmed by agarose gel electrophoresis.

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L6: Entry 24 of 37 File: PGPB May 30, 2002

DOCUMENT-IDENTIFIER: US 20020064515 A1
TITLE: Methods and products for stimulating the immune system using immunotherapeutic oligonucleotides and cytokines

Detail Description Paragraph:
[0097] Lipid formulations for transfection are commercially available from QIAGEN, for example as EFFECTENE.TM. (a non-liposomal lipid with a special DNA condensing enhancer) and SUPER-FECT.TM. (a novel acting dendrimeric technology) as well as Gibco BRL, for example, as LIPOFECTIN.TM. and LIPOFECTACE.TM., which are formed of cationic lipids such as N-[1-(2, 3 dioleyloxy)-propyl]-N, N, N-trimethylammonium chloride (DOTMA) and dimethyl dioctadecylammonium bromide (DDAB). Methods for making liposomes are well known in the art and have been described in many publications. Liposomes were described in a review article by Gregoriadis, G., Trends in Biotechnology 3:235-241 (1985), which is hereby incorporated by reference.

Detail Description Paragraph:
[0114] Preferably the CpG oligonucleotide is in the range of between 8 and 100 and more preferably between 8 and 30 nucleotides in size. Alternatively, CpG oligonucleotides can be produced on a large scale in plasmids and degraded into oligonucleotides.

Detail Description Paragraph:
[0115] The CpG oligonucleotide and immunopotentiating cytokine may be directly administered to the subject or may be administered in conjunction with a nucleic acid delivery complex. A "nucleic acid/cytokine delivery complex" shall mean a nucleic acid molecule and/or cytokine associated with (e.g. ionically or covalently bound to; or encapsulated within) a targeting means (e.g. a molecule that results in higher affinity binding to target cell (e.g. dendritic cell surfaces and/or increased cellular uptake by target cells). Examples of nucleic acid/cytokine delivery complexes include nucleic acids/cytokines associated with: a sterol (e.g. cholesterol), a lipid (e.g. a cationic lipid, virosome or liposome), or a target cell specific binding agent (e.g. a ligand recognized by target cell specific receptor). Preferred complexes should be sufficiently stable in vivo to prevent significant uncoupling prior to internalization by the target cell. However, the complex should be cleavable under appropriate conditions within the cell so that the nucleic acid/cytokine is released in a functional form.

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L6: Entry 27 of 37 File: PGPB Dec 6, 2001

DOCUMENT-IDENTIFIER: US 20010048939 A1
TITLE: CATIONIC REAGENTS OF TRANSFECTION

Summary of Invention Paragraph:
[0003] Compared to other methods, liposome-mediated transfection is characterized by high reproducibility, low cytotoxicity and simple procedures. However, many cationic compounds useful for liposome-mediated transfection are based on ester-linkages and are rapidly degraded by hydrolysis. Compared to infectious agents, cationic liposomes often show low overall efficiencies. Moreover, the commercially available cationic liposomes cannot be used or adapted for transfection of specific subpopulations of cells either in vitro or in vivo.

Summary of Invention Paragraph:
[0088] In pharmaceutical formulations, the compounds of the present invention may be used in those contexts where cationic lipids are acceptable for the formulation of creams, pastes, gels, colloidal dispersions, and the like. For additional information, reference is made to Remington's Pharmaceutical Society, 17th Edition, Mark Publishing Company, Easton, Pa. (1985), or any other standard treatise on pharmaceutical formulations.

Summary of Invention Paragraph:
[0091] In another embodiment, the CpG motifs are inserted into a plasmid DNA vector, said vector is then replicated in a bacterial cell, allowing the CpG motifs to retain their unmethylated form. Said vector, or parts thereof, is then harvested and delivered to a target cell by the liposomes of the present invention, as an immunostimulatory substance, or together with a vaccine as an adjuvant.

Summary of Invention Paragraph:
[0103] Liposomes comprising exogenous compounds, for example biologically active substances, may be formulated into compositions suitable for administration. For example, for oral administration, a compound of the present invention may be given in the form of a capsule, tablet, or gel. In other embodiment, a compound of the present invention may be given in the form of an ointment, salves, gel, cream, patch, or suppository. The compounds of Formula (I) are particularly useful in the preparation of liposomes, but may be used in any of the many uses for which cationic lipids find application. For example, they may be used in industrial applications, in food or feeds, in pharmaceutical formulations, cosmetic compositions, or other areas where lipids may be employed.

Full - [FULL]
Title - [TI]
Citation - [CIT]
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Review - [REV]
Classification - [CLAS]
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Reference - [REF]
Sequences - [SEQ]
Attachments - [ATT]
Claims - [CLM]
KWIC - [KWIC]
Drwg Desc - [DRAW]
Image - [IMG]

CLAIMS:

15. Use of a compound according to claim 13 or 14, characterized in that the DNA is selected form the group of plasmids, vectors, cDNA, CpG-motifs, and/or oligonucleotides, and the RNA is selected from the group of mRNA, oligonucleotides or ribozymes.

L6: Entry 29 of 37 File: USPT Aug 6, 2002

DOCUMENT-IDENTIFIER: US 6429199 B1
TITLE: Immunostimulatory nucleic acid molecules for activating dendritic cells

Detailed Description Text (29):
Preferably the immunostimulatory CpG DNA is in the range of between 8 to 30 bases in size when it is an oligonucleotide. Alternatively, CpG dinucleotides can be produced on a large scale in plasmids, which after being administered to a subject are degraded into oligonucleotides. Preferred immunostimulatory nucleic acid molecules (e.g. for use in increasing the effectiveness of a vaccine or to treat an immune system deficiency by stimulating an antibody (i.e. humoral response in a subject) have a relatively high stimulation index with regard to B cell, dendritic cell and/or natural killer cell responses (e.g. cytokine, proliferative, lytic or other responses).

Detailed Description Text (30):
A "nucleic acid delivery complex" shall mean a nucleic acid molecule associated with (e.g. ionically or covalently bound to; or encapsulated within) a targeting means (e.g. a molecule that results in higher affinity binding to target cell (e.g. dendritic cell surfaces and/or increased cellular uptake by target cells). Examples of nucleic acid delivery complexes include nucleic acids associated with: a sterol (e.g. cholesterol), a lipid (e.g. a cationic lipid, virosome or liposome), or a target cell specific binding agent (e.g. a ligand recognized by target cell specific receptor). Preferred complexes must be sufficiently stable in vivo to prevent significant uncoupling prior to internalization by the target cell. However, the complex should be cleavable under appropriate conditions within the cell so that the nucleic acid is released in a functional form.

Detailed Description Text (71):
When the vaccine is a DNA vaccine at least two components determine its efficacy. First, the antigen encoded by the vaccine determines the specificity of the immune response. Second, if the backbone of the plasmid contains CpG motifs, it functions as an adjuvant for the vaccine. Thus, CpG DNA acts as an effective "danger signal" and causes the immune system to respond vigorously to new antigens in the area. This mode of action presumably results primarily from the stimulatory local effects of CpG DNA on dendritic cells and other "professional" antigen presenting cells, as well as from the co-stimulatory effects on B cells.